Development and Evaluation of Polyherbal Formulations Against MDR Bacteria *Staphylococcus aureus* Causing Skin Disease.

A Synopsis Submitted in Partial Fulfilment for the Degree

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Introduction

Skin reflects the internal health of an individual and a healthy skin depicts the personality of an individual. Skin diseases are becoming very common nowadays. Various causes like consumption of un healthy food, lifestyle modification, improper day schedule, environmental pollution or due to stress skin problems are increasing day by day and it also affects the quality of the life of an individual**[1].** The skin is one the most extensive organs of the human body covering an area of about $2m^2$ in an average human adult. The skin separates the underlying blood circulation network from the outside environment and performs the following functions.

- Serves as a barrier against physical, chemical and microbial attacks.
- Protects against invaders, environmental danger, foreign substances
- Protect the body from water loss .
- Acts as a thermostat in maintaining body temperature
- Protects against harmful ultraviolet rays of the sun
- Plays a role in the regulation of blood pressure

Anatomically, the skin has many histological layers but in general, it is described in terms of three major tissue layers: epidermis, dermis and hypodermis. The barrier function of the skin is located in the superficial layer of the skin, the stratum corneum. This layer consists of dead cells filled with keratin and water. At the surface of the skin, already present microorganisms grow as small colonies and constitutes various non-pathogenic bacteria, fungi and viruses. However, when cutis is exposed to pathogenic microorganisms and to combat this threat the residential cells of the epidermis evolves several preventive mechanisms **[2].** Apart from that still there are many skin diseases which can be caused by one or the other factors. The most common skin diseases are Alopecia's, tania infection, premature grayness or whiteness of the scalp hair (Canities), These diseases are encountered in day to day practice. Now awareness regarding the skin health is increased as compare to past. Apart from these diseases various other diseases involves skin infections which are caused by bacteria as well as fungi are acne , Pimples, Eczema, Impetigo, Folliculitis, Scabies, Chloasma, Urticaria, Atopic & Contact Dermatitis, Paronychia, Kerato Derma, Corn etc. Microbial diseases of the skin are usually transmitted by contact with an infected individual. Although the skin normally provides a barrier to infection but when it is penetrated by microorganisms and infection develops. Among all others the most common agent causing skin diseases includes *Staphyllus species*.

Staphylococci are Gram-positive cocci occurring in clusters. And the most commonly known is *Staphylococcus aureus*. This organism invades the hair follicles and causes folliculitis, also referred to as pustules if it is deeper, the infection of the skin tissues is referred to as a boil, abscess or furuncl**e** usually filled with pus. A large lesion progressing from a boil is known as a carbuncle**.** Infections such as these are easily transmitted by skin contact and toxin-producing strains of *S. aureus also* causes scalded skin syndrome**.** Usually found in young children and babies. It is characterized by vesicles on the body surface, which cause the skin to peel and give a scalded appearance. Penicillin or erythromycin antibiotics are used to treat this and other staphylococcal skin diseases but extensive use of antibiotics are leading to multiple drug resistance**[3].**The ability of microorganisms to become resistant to the major therapies used against them is called antimicrobial drug resistance and if this resistance occurs for one more than two antibiotics it is termed as multiple drug resistance. The chief contributors of antimicrobial resistance problem are the hospitals and thus increasing antimicrobial resistance (AMR) is developing a major threat to public health because it reduces the effectiveness of antimicrobial treatment which may lead to increased morbidity, mortality, and health care expenditure **[4,5]**

Drug resistant strains are causing severe problems in many infections including skin infections such as carbuncles, folliculitis, impetigo, and burn wound sepsis.Because of antimicrobial resistance.Infectious diseases are difficult to control globallyand the effect of antibiotics action is being reduced by the appearance of multidrug-resistant (MDR) pathogens. **[6]**. One of the ways to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. Apart from the problem of resistance, environmental degradation, cost and pollution associated with irrational use of orthodox medicines have necessitated renewed interest in nature as a source of effective and safer alternatives in the management of human infections **[7].**

The rate of skin infections has becomea significant health problem in many underdeveloped and developing countries and is particularly predominant in overpopulated areas with high humidity and poor hygienic conditions **[8]**. For treatment of skin diseases conventional drugs like streptomycin, Aclometasone, Allantoin ,Amcinonide, Cefdinir , Clobetasolepropinate, Econazole, Gatifloxacin, Hydroquinone, Isotretinoin, Metronidazole, Neomycin, Ofloxacin, Clarithromycin, Vancomycin are given to the patients but because of the dangers of

allopathic drugs like adverse effects, hypersensitivity reactions, neurological effects like insomnia, headache, breathing problems, rashes, inflammation, blistering of skin, swelling of face, tongue, lips& throat, diarrhoea, vomiting, flatulence, kidney disease, asthma are resulting in limited use of allopathic drugs.Along with increasing side effects day by day antimicrobial resistance is also increasing . Hence this increasing resistance and risk of adverse effects of chemical agents have given rise to use of herbal medicines. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines so they are found to be a good source of secondary metabolites which are chief source of valuable bioactive compounds and drugs**.[9]**

Plant-derived substances have recently attracted great interest, owing to their versatile applications like in modern medicine as nutraceuticals and food supplements, pharmaceutical intermediates, and chemicalentities for synthetic drugs **[10].**Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverseeffects. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms.The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants. **[11].** Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products used in the traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced in the market are obtained from natural or semi synthetic resources**[12].** In India, from ancient times, different parts of medicinal plants have been used to cure specificailments. Several medicinal plants have been used since time immemorial for treatment of cuts, wounds and burns and showed promising effects. Some very common plants like *Aloe vera* , *Azadirachtaindica* , *Carica papaya* , *Celosia argentea* , *Centella asiatica* , *Cinnamomumzeylanicum* , *Curcuma longa* , *Nelumbonucifera* , *Ocimum sanctum* , *Phyllanthusemblica* , *Plumbagozeylanica* , *AdhatodavasicaPterocarpussantalinus* , *Terminalia arjuna* and *Terminalia chebula* have been extensively reported in Ayurveda, Siddha and Unani systems of medicines for their wound healing potential.Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment

of diseases but are also often with adulteration and side effects so herbal remedies for skin care with antibacterial and antifungal activities are prepared from variety of plant parts such as leaves, stem, bark and fruits. These medicines are administered topically and may be applied in the form of cream, lotion, soap, solvent extract and ointment and have been established to possess antimicrobial properties. The route of drug delivery through the skin has been a promising concept because of the ease of access, large surface area, vast exposure to circulatory and lymphatic system. **[13].**

When two or more herbs are used for the formulation it is known as polyherbal formulation. Drug formulation in *Ayurveda* is based on two principles: Use as a single drug and use of more than one drugs, in which the latter is known as PHF. This key traditional therapeutic herbal strategy exploits the combining of several medicinal herbs to achieve extra therapeutic effectiveness, usually known as polypharmacy or polyherbalism.

Advantages of polyherbal formulations:

1. Fewer side effects as compared to allopathic drugs. Although modern allopathic drugs are designed for efficacious therapeutic results, administration of most of them come with unwanted side-effects
2. Safe to use.
3. Express high effectiveness in a vast number of diseases
4. No drug resistance observed.
5. Have wide therapeutic range. Most of them are effective even at a low dose and safe at high dose, thus they have superior risk to benefit ratio.
6. Product of the nature, they are relatively cheaper, eco-friendly and readily available than allopathic drugs. Their better affordability and greater accessibility account for increasing demand globally, especially in rural areas and some developing countries, where costly modern treatments are not available.

   All the above reasons: Effectiveness, safety, cheap, ubiquity and better acceptance, made PHF an ideal treatment of choice, hence higher compliance by the patients and excellent therapeutic effect is ensured.

Major problems related to Poly Herbal Formultion usage

Despite the fact that *Ayurvedic* PHFs are beneficial to mankind in many aspects but still they challenged by some unavoidable drawbacks, affecting their ability and efficacy in treatments. These problems lie within the PHFs' sources and manufacturing process patients, *Ayurvedic* practitioners, as well as the law and regulations.

We cannot say that *Ayurvedic* PHFs are always safe. *Charaka Samhita* itself has described that *Ayurvedic* medicines have adverse effects when prepared or used inappropriately. The concurrent use of PHFs with allopathic drugs is increasing as most of the individual patients do not inform their medical practitioners on the concomitant treatments.

- **Brief Description of selected plants**

1. ***Murrayakoenigii:*** The plant *Murraya koenigii* (*Rutaceae)* has a versatile role to play in traditional medicines. It is popularly known as Curry patta and is popular leaf-spice used in very small quantities for their distinct aroma due to the presence of volatile oil and used to improve digestion, in diarrhea & also used as better alternative in food preservation. A paste made of curry leaves is can also be used on boils for quick relief. Along with mint leaves and coriander leaves, curry leaves can be used in treating excessive pitta conditions. It is effective in burn, bruises and skin eruption. Fresh juice of curry leaves prevents cataract& juice of roots for curing kidney pain. **[14,15]**
2. ***Dodonaea viscosa*:** This plant belonging to family Sapindaceae is an evergreen shrub widely distributed almost throughout the world and is known by several names such as Kitkita (in Amharic), Aliar, Bandaraaku and others *D. viscosa*is reported to possess several medicinal properties. It is used as an antipruritic and for the treatment of sore throat, dermatitis and hemorrhoids and for treatment of skin lesions , in rheumatism, skin infections, diarrhea and disorders involving smooth muscles as well as for women's health to treat menorrhagia, infertility, irregular bleeding and to prevent miscarriage **[16]**
3. ***Mentha spicata:*** belonging to family Lamiaceae contain essential oil together with apigenin, hesperidin, chlorogenic acid, ursolic and oleanolic acid along with flavonoids. Most of its

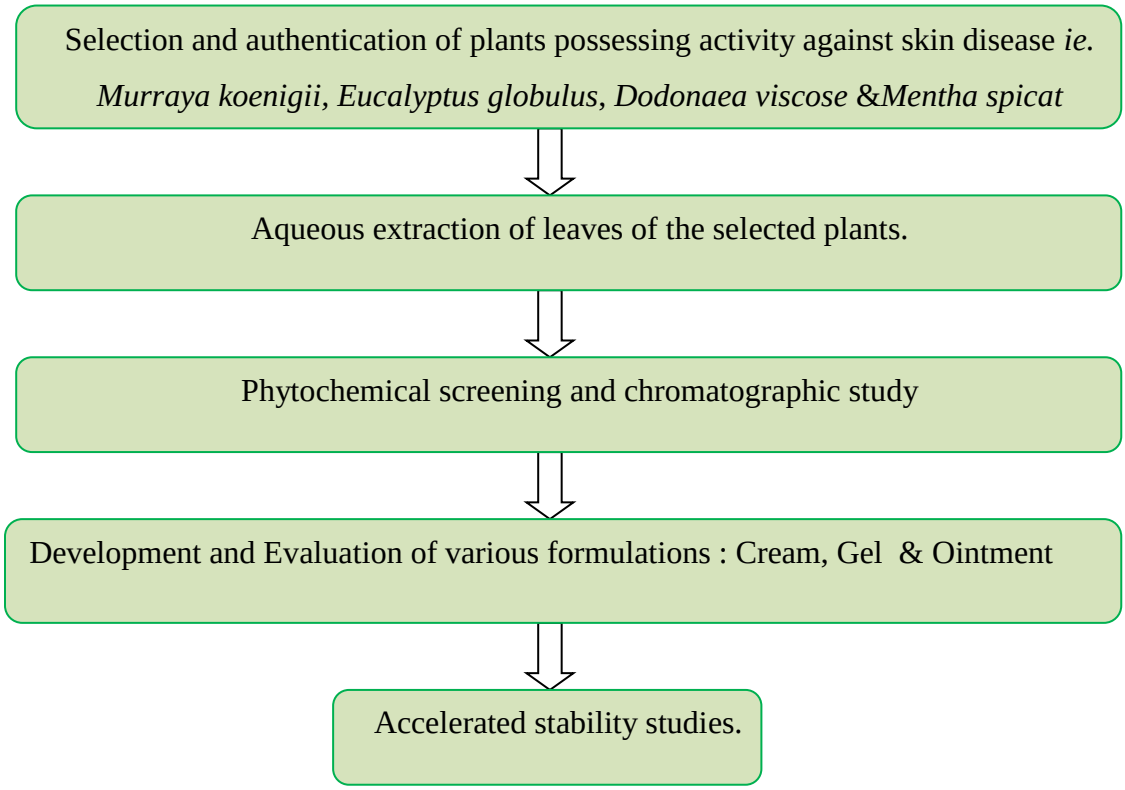
biological acitivity is due to the presence of all these phytochemicals. It is used as antispasmodic, cholagogic, antiseptic, astringent, diuretic, and antioxidant effect and antiseptic effect **[17,18]**

4. **Eucalyptus globules:** Eucalyptus plant belonging to the Myrtaceae family. Commonly known as Safeda. The products of this tree have multifold uses i.e. medicinal as well as insecticide /herbicide/ pesticides, even the plant waste stem bark, that is shed periodically can be utilized as food dye with high antioxidant activity. Its leaf extract reduce serum triglyceride and cholesterol . It also contain volatile oils which are classified into three types; medicinal, perfumery and industrial. Leaf extract and essential oil of Eucalyptus sp. have antifungal, repellent, antibacterial, analgesic and anti-inflammatory activities.**[19]**

The above mentioned four important herbs have reported significant anti-bacterial and anti inflammatory activities which are complementary to skin disease treatment. The growing popularity of natural and herbal medications, easy availability of raw materials, cost effectiveness and paucity of reported adverse reaction, prompted us formulate a poly herbal topical preparation of anti-microbial activity. Thus here is an attempt for the formulation of poly herbal ointment having both the effects.

Objective Of Study:

The main objective therefore is to find out and confirm the phytoconstituents in the extracts of these plants and develop polyherbal formulations which can be topically used to treat skin disease caused by MDR bacteria. Specific objectives include:


Selection and authentication of plants possessing activity against skin disease *ie. Murraya koenigii, Eucalyptus globulus, Dodonaea viscose &Mentha spicat*
Aqueous extraction of leaves of the selected plants.
Phytochemical screening and chromatographic study
Development and Evaluation of various formulations : Cream, Gel & Ointment
Accelerated stability studies.

Review of Literature

- **Skin and its diseases:**

1. **Anjana (2014)** studied concept of kshudraroga in ayurveda''with special reference to skin diseases. The review was on the basis of ayurveda and it discussed etiology, features and treatment with special reference to skin and its diseases under kshusdraroga . It revealed that skin disease or Kshudraroga have become very common these days and the main cause are: consumption of unhealthy food, life style modifications, pollution and stress.in this review ayurvedic books and various types of skin diseases are groups in various categories and are breifly discussed **[1]**

- **Antimicrobial herbs for MDR bacteria:**

1. **Kirti rani *et al* ., (2016)** studied a comprehensive brief review on antimicrobial herbs and spices. In this paper a detailed review on various herbs and spices having antimicrobial activity is been discussed. As ancient herbs are cost effective and have potent antimicrobial action hence, list of various herbs are given which can be used for microbial infections. Various herbs discussed in this review are :Elettaria&Amomum(Cardamom), Cuminumcyminum (Cumin), Aloe barbadensis(Aloe vera), Curcuma longa (Turmeric), Syzigiumaromaticum (Clove), Crocus sativus (Saffron), Foeniculum vulgare (Fennel), Azadirachtaindicum (Neem) **[20]**
2. **Mishra *et al* ., (2015)** performed *In vitro* antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria . Examination of 9 tropical flowering plants (Anogeissusacuminata, Azadirachtaindica, Bauhinia variegata, Boerhaaviadiffusa, Punicagranatum, Soymidafebrifuga, Terminalia chebula, Tinosporacordifolia and Tribulusterrestris) for possible use as source of antimicrobials for multidrug resistant (MDR) bacteria, along with main-stream antibiotics was done . Pathogenic bacteria were isolated from urine samples of patients attending and admitted in the hospital. Antibiograms of 11 isolated bacteria (GPs, Enterococcus faecalis and *Staphylococcus aureus*; and GNs, Acinetobacter baumannii, Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris and

Pseudomonas aeruginosa) were ascertained by the disc-diffusion method, and antibacterialeffectivity of plant extracts was monitored by the agar-well diffusion method. Isolated bacteria were floridly MDR to most antibiotics of the day. Methanol extracts of 9 plants were used, and extracts of 3 plants, A. acuminata, P. granatum and S. febrifuga at least caused 25–29 mm as the maximum size of zone of inhibition on bacterial lawns. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of methanol extracts of 9 plants were recorded. The methanol extract of A. acuminata had 0.29 mg/ml as the lowest MIC value and 0.67 mg/ml .**[21]**

3. **Sharmeen *et al* ., (2012)**worked on *In-vitro* antibacterial activity of herbal aqueous extract against multi-drug resistant *Klebsiella* sp. isolated from human clinical samples. The studies were carried out to evaluate antibacterial activity of 35 aqueous herbal extracts against a total of 20 clinical *Klebshiella*sp. isolates. The maximum antibacterial activity was found as 90% in crude extracts of *Syzygiumaromaticum*(leaf) and *Citrus limon*L. (fruit) followed by 85% in *Spondiaspinnata*(leaf). Sensitivity of these isolates was also evaluated for eight commercial antibiotic discs following disc diffusion assay where most of the isolates found to develop resistance against multiple commercial antibiotics. 85% of isolates exhibited resistant to chloramphenicol and erythromycin and 80% were resiatant to sulfamethoxazole and cephradine. The isolates showed their resistance between 55-60 % to the other four antibiotic discs, *viz*; gentamycin, streptomycin, ciprofloxacin and azithromycin. Among 35 herbal extracts tested, 19 herbal extrats were found to possess antimicrobial activity in all multi-drug resistant isolates. Therefore these herbal extracts could be used in future direction as alternative therapeutic agents for the treatment of human diseases caused by *Klebsiella* sp.**[22]**

4. **Khan *et al* .,(2009)** performed Antimicrobial Activity on five herbal extracts against multi drug resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. Antimicrobial activities of the crude ethanolic extracts of five plants werescreened against multidrug resistant (MDR) strains of *Escherichia coli, Klebsiellapneumoniae* and *Candida albicans.* ATCC strains of *Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, Streptococcus bovis, Pseudimonas aeruginosa, Salmonella typhimurium, Escherichia coli,*

Klebsiella pneumoniae and *Candida albicans*were also tested. The strains that showed resistance against themaximum number of antibiotics tested were selected for an antibacterial assay. The MDRnstrains were sensitive to the antimicrobial activity of *Acacia nilotica, Syzygium aromaticum* and *Cinnamum zeylanicum*, whereas they exhibited strong resistance to the extracts of *Terminalia arjuna*and *Eucalyptus globulus*. Community-acquired infectionsshowed higher sensitivity than the nosocomial infections against these extracts. The mostpotent antimicrobial plant was *A. nilotica* (MIC range 9.75-313μg/ml), whereas other crude plant extracts studied in this report were found to exhibit higher MIC values than *A. nilotica* against community acquired as well as nosocomial infection. This study concludesthat *A. nilotica, C. zeylanicum* and *S. aromaticum*can be used against multidrug resistantmicrobes causing nosocomial and community acquired infections. **[23]**

- **Herbal Antimicrobial formulations**

1. **Ezeadila Joachim O *et al* ., (2016)** performed research on *in-vitro* antibacterial activity of crude citrus extracts on *staphylococcus aureus* and *escherichia coli* isolated from the human skin and stool respec. 50 skin swab and rectal swab samples were randomly collected from some students of NnamdiAzikiwe University, Awka and processed by culturing them on suitable media. The antibacterial activities of crude extracts of different Citrus fruits were determined using the disc diffusion method. The results obtained confirmed antibacterial activity of extracts of Citrus fruits with inhibition zone diameters comparing to those of the commercial antibiotic discs. The highest zone of inhibition (19mm) was observed with the crude extract of *Citrus limon*and followed by *Citrus aurantifolia*(17mm) on *Staphylococcus aureus,* with a minimal effect on *Escherichia coli* producing zone of 11mm and 13mm respectively. The crude extract of *Citrus paradisi*produced a zone of inhibition of 11mm on *Staphylococcus aureus*, whereas *Escherichia coli* was resistant to it. The two organisms were both resistant to the crude extracts of *Citrus sinensis.* Hence this study suggested that Citrus extracts especially those of *Citrus limon*and *Citrus aurantifolia*show great antibiotic potential against *Staphylococcus aureus* and *Escherichia coli* and thus promise an efficacious substitute of synthetic antibiotics. **[24]**

2. **Adapa Satish Kumar *et al* ., (2016)** carried out his study on formulation and evaluation of antibacterial activity of a topical antifungal herbal gel containing ethanolic extract of *cassia alata*leaves.Various formulations of *Cassia alata*, have been formulated by with carbopol 940 and other excipients by adding 0.1%, 0.2 % & 0.3 % *Cassia alata*ethanolicleaf extract and the extract with highest concentration showed the maximum effect and the rest two were considered safe and stable.Evaluation parameters like physical appearance, consistency, greasiness, odour, extrude ability, homogeneity, pH, viscosity, and anti-bacterial activity were also performed. Finally the study reveals that application of topical gels offer great advantage in a faster release of drug directly at the pathological site, independent of solubility of the drug when compared to creams and ointments **[25]**
3. **Kamarapu P *et al* ., (2015).** Thisresearch involvesFormulation and evaluation of anti-microbial polyherbal ointment was done by using methanolic extracts of Shatavari, Terminalia chebula and were evaluated for its physicochemical properties. The methanolic extracts of the chosen plants were taken in specific ratio in emulsifying ointment as a base and the antimicrobial tests of the combinations were applied. physicochemical properties like loss on drying, pH, spread ability, extrudability and gave satisfactory results. Probably the extract mixture with the highest zone of inhibition on *Escherichia coli, Staphylococcus aureus* was the most powerful one. The entire formulations were found to be very efficacious in all the parameters which has conducted and also found enhance antimicrobial property**.[26]**
4. **Stephen olaribigbe *et al.*, (2014):** performed his study to explore the antifungal and antibacterial activity of methanolic extract of Cassia alata(C. alata) and its formulations for skin diseases. Leaves of C. alataLinn. were extracted using different solvents like water, methnol, ethanol, n-hexane and acetone.theobtaines extracts were investigated for antibacterial and anti fungal activities using disc diffusion methods againdCoccidioidesimmitis, Exophiliadermatitidis , Aspergillus fumigates and human pathogenic fungi Candida albicansand a group of bacteria, *Staphylococcus aureus* . The minimum inhibitory concentrations was determined using agar dilution methods. Herbal ointmenrs were prepared using by incorporating methanol extract of C. alata into emulsifying ointment to obtain different concentrations of 25, 50, 100, and 200 mg/mL. As a result The methanol extraction gave the maximum extraction and the formulatedC. alata ointment when compared with standard nystatin and streptomycin in vitro was more effective. Hence thestudy showed that C. alataointment had good antifungal and antibacterial effects. **[27]**

5. **Mishra *et al* .,(2014) c**arried out the study on preparation and evaluation of herbal antioxidant face cream made of ethanolic extract of *Nardostachys jatamansi* . In this research anti oxidant activity of ethanol extract was assessed by previously reported 2,2 diphenyl, 1- picrylhydrazyl method and later different types of formulations i.e. oil in water were prepared to create several face creams namely F1 to F6 by . incorporating different concentrations of stearic acid and acetyl alcohol. Evaluation of all formulation (F1 to F6) was done by analysis of different parameters like pH, viscosity and spread ability. An ethanolic fraction of *Nardostachysjatamansi* showed significant antioxidant activity with an IC_{50} value of 58.39µg/mL while for Ascorbic acid it was 46.68 µg/mL.Among six formulations (F1 to F6) F5 & F6 showed good spread ability, good consistency, homogeneity, appearance, ph and no phase separation . Also F5 and F6 showed no redness, edema, itching and irritation during irritancy studies**. [28]**

6. **Charde *et al ., (2014) w***orked on development and evaluation of herbal gel for the treatment of acne .The ethanolic extract of Neem (leaves), Nutmeg (fruit), and Black pepper (fruit) were prepared and formulated into a topical gel. In vitro antibacterial activity was performed against *P. acnes, S. epidermidis* and *S. aureus,* using agar well diffusion method. The measured zones of inhibitions of the prepared formulations were compared with standard antibiotic (Clindamycin) and standard marketed topical herbal formulation. The prepared gels were evaluated for pH, viscosity, spreadability, stability, drug content, acute skin irritancy activity and *in vitro* diffusion. The results from the agar well diffusion showed that Neem, Nutmeg and Black pepper inhibited the growth of *P. acnes, S. epidermidis* and *S. aureus* and the prepared polyherbal gels showed comparable antimicrobial activity against these bacterias**.[29]**
7. **Rajasree *et al* .,(2012**) prepared and evaluated polyherbal antiseptic ointment. Ointmentsusingmethanolic extracts (by continuous hot percolation-soxhletation) of *Azadirachtaindica*, *Chromolaenaodorata*, *Mimosapudica*, *Samaderaindica* and were evaluated for its physicochemical property, antibacterial and antioxidant activity. Ointments were prepared using different concentrations of the extracts such as 2%, 4%, 6% w/w by fusion method using emulsifying ointment as base and then tested for its physicochemical properties like loss of drying, pH, spreadability, extrudability and diffusion study. The prepared formulations gave satisfactory results and were also stable at 4ºC, 25ºC and 37ºC.

Further, polyherbal formulations were evaluated for its anti-bacterial activity against *Staphylococcus aureus*, Pseudomonas sp., Bacillus sp., by agar diffusion method by using Betadine (5%w/w) as the standard and MIC was determined. All the formulations showed predominant activity against selected species. Formulations were also evaluated for antioxidant activity through reducing power assay, nitric oxide and hydrogen peroxide scavenging method. The scavenging activity of the formulations increased with increase in concentration .hencedueo to both antibacterial and antioxidant activity the prepared ointment was said be used for wound healing. **[30]**

8. **Prasanna A Datar (2013)** worked on Formulation and Evaluation of the Polyherbal Gel for Treating Skin Diseases in Comparison with Ointment . Gel was formulated using herbal extracts of neem , aloe, tulsi and fenugreek and gelling agent as Carbopol 934 in comparison with emulsifying ointment prepared by taking same herbal drugs using emulsifying ointment base B. P. The gels and ointments were evaluated for various physicochemical parameters like pH, viscosity, spread ability, skin irritation test and microbial evaluations. Among the various formulations prepared for each of the gel and ointment, increasing concentrations of each of the herbal extracts were used from 1 to 10% w/w and thereof these compositions having concentrations 2 to 5% w/w gave best results and in microbial evaluation the zone of inhibition of the gel showed greater diffusion and inhibition for gel formulation composed of 5% herbal extract than ointments. **[31]**

- **Anti microbial study of selected plants**

1. **Akula *et al* ., (2016)** performed the evaluation of anti-microbial activity of leaf and bark extracts of *Murrayakoenigii*(curry leaves)**.** Preliminary phytochemical screening was performed for Ethanolic leaf and bark extracts and results showed the presence of carbohydrates, alkaloids, tannins & saponins. These extracts were screened for their anti-microbial activity against the pathogens namely *Staphylococcus aureus, Proteus vulgaris, Enterobacter aerogenes & Lactobacillus* by agar cup-plate method. The pattern of inhibition varied with both the extracts and the microorganism tested. Among these extracts, Ethanolic bark extract showed the significant anti-bacterial activity against most tested microbes. The

most susceptible micro-organism was *Staphylococcus aureus* followed by Proteus vulgaris and Enterobacter aerogenes. **[32]**

2. **Singh V *et al* ., (2013)** worked on an antibacterial formulation comprising water extracts of *Murraya koenigii* for killing of bacteria. The formulation was reported to be effective against Vancomycin resistant *Staphylococcus aureus* (VRSA) bacteria.**[33]**
3. **Amabye *et al* ., (2016)** worked on evaluation of Phytochemical and Antimicrobial Potentials Leaves Extract of *Eucalyptus Globulus* oil from Maichew Tigray Ethiopia . The extracts for phytochemical screening and antimicrobial activity were carried out on dried fresh leaves, *Eucalyptus globules* using water as solvent. The results of the phytochemical screening showed that the plant parts contained saponins, tannins, phenols and glycosides. The disc diffusion method was adopted for the antimicrobial activity of the plant extracts. The antimicrobial activity test of the plant extracts on Escherichia coli, *Staphylococcus aureus*, Salmonella typhi and Bacillus subtilis showed that water extracts had inhibitory activity on all the tested organisms. The results obtained from this study revealed that extracts of *Eucalyptus globules* possess antimicrobial activities against some microorganism that causes diseases. **[34]**
4. **Pirzada *et al* ., (2013)** worked on antifungal activity of *dodonaea viscose* acq extract on pathogenic fungi isolated from super ficial skin infection. Antimicrobial activity ofleaves and shoot extract of *Dodonaea viscose* J acq using various solvents have been determined against fungi, *Aspergillus niger*, *Aspergillus flavus, Paecilomycesvarioti, Microsporumgypseum, and Trichophyton rubrum* causing skin diseases. All crude extracts were found to be effective against tested fungi. However chloroform has strong inhibition activity against fungi as compared to ethanol, methanol, ethylacetate and aqueous extracts. Some basic elements were also analyzed, Al, Ca, Cu, Fe, Mg, Mn, P, S and Zn from the medicinal plant *Dodonaea viscosa* J acq. by using atomic absorption. spectrophotometry and UV spectrophotometry. **[35]**
5. **Padhy *et al* ., (2014)** monitored in vitro antibacterial efficacy of 26 Indian spices against multidrug resistant urinary tract infecting bacteria . Screening of methanolic extracts of 26 commonly used Indian spices against nine species of uropathogenic bacteria (Enterococcus faecalis, *Staphylococcus aureus*, Acinetobacter baumannii, Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, and Pseudomonas

aeruginosa), isolated from clinical samples of a tertiary care hospital for antibacterial activity was done. Bacterial strains were subjected to antibiotic sensitivity testing by Kirby–Bauer's disc diffusion method. Antibacterial potential was monitored of various spice extracts by the agar-well diffusion method with multidrug resistant (MDR) strains of nine uropathogens.The Gram-positive (GP) bacteria E. faecalis and S. *aureus* were resistant to 16 of the 21 antibiotics used. Among the Gram-negative (GN) bacteria, resistant patterns were A. baumannii and E. aerogenes to 12, C. freundii to 14, E. coli to 12, K. pneumoniae to 10, P. mirabilis to 11, and P. aeruginosa to 15 antibiotics of the 18 antibiotics used. The most effective 15 spices, having at least 25–29mm as the size of the zone of inhibition, were Allium cepa, Brassica juncea, Cinnamomumtamala, Cinnamomumzeylanicum, Coriandrumsativum, Cuminumcyminum, Curcuma longa, Menthaspicata, Murrayakoenigii, Nigella sativa, Papaver somniferum, Piper nigrum, S. aromaticum, Trachyspermumammi, and Trigonellafoenum for at least one of the GP or GN MDR bacterial strains used. Finally study revealed that the best two spices for controlling all the pathogens used were C. zeylanicum and C. longa.**[36]**

RESEARCH METHODOLGY

Materials & Methods:

- Collection & Authentication of plants:
- Leaves of *Murraya koenigii*, *Eucalyptus globulus*, *Dodonaea viscose* &*Mentha spicata*
- Monographic analysis of herbs
  Ash value, Extractive value, Foreign matter, LOD, Moisture content.
- Heavy metal analysis
- Preparation of aqueous extracts of leaves of the selected plants
- Qualitative phytoprofiles of extracts: Chemical tests for alkaloid, flavonoid, tannins, glycosides, phenolic compounds, carbohydrates, saponins.
- Chromatographic study ; TLC & HPLC
- Evaluation of in vitro anti-bacterial activity of extracts; By Agar Well Diffusion method
- Development of various formulations :

- **Cream** : Oil in water emulsion based cream .

  Oil phase- stearic acid, cetyl alcohol, almond oil

  Aqueous phase- (methylparaban, propyl paraban, triethanolamine, propylene glycol and Aqueous Plant extracts
- **Gel** : Carbopol 934. Triethanolamine was used as a neutralizer, Benzoic acid ,Aq. Plant extracts
- **Ointment**: will be prepared by fusion method using emulsifying ointment base B.P. soft paraffin, emulsifying wax and liquid paraffin , Aq. Plant extracts.

➢ Evaluation parameters of each preparedformulation:- Cream, Gel and Ointment will be evaluated for various physicochemical parameters like pH, viscosity, spreadability, skin irritation test and microbial evaluations.

➢ *In vitro & In vivo* studies for anti-bacterial activity of various formulations against *Streptococcus aureus*

➢ Accelerated stability studies.

ACTIVITY PLAN

1st semester

- Finalize topic & searching literature - 6 months
- Synopsis Preparation - 3 months

2nd and 3rd semester

- Procurement &authentication of crude drug and collection of excipients &extensive research work submission of at least research paper in journal of repute - 10 months

4th semester

- Compilation of data - 3 months
- Writing of thesis work - 2 months
- Submission of thesis - 1 month

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